Minireview

Bacterial sources and sinks of isoprene, a reactive atmospheric hydrocarbon

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Introduction

Physiological and biochemical processes in bacteria can have important impacts on the atmosphere. For example, methane is produced by anaerobic methanogens and then released from soils, sediments and ruminant animals to contribute to the atmospheric 'greenhouse' effect (Crutzen, 1991; Conrad, 1996). Interestingly, it is estimated that the fraction of methane that reaches the atmosphere is only about 10% of that formed in anaerobic environments – aerobic methanotrophs in soils and sediments and associated with plant roots are very adept at scavenging methane before it can be released to the atmosphere (King, 1992; Hanson and Hanson, 1996). Methanotrophs can also take up methane from the atmosphere, although the magnitude of this sink is unclear (Potter *et al*., 1996). Thus, the concentration of one of the most important trace gases in the atmosphere is controlled in part by the balance between bacterial methane producers and consumers. As reviewed by Conrad (1996), similar patterns of bacterial producers and consumers control emissions of other important atmospheric trace gases, such as CO, NO and N_2O.

From recently reported work, it appears that yet another bacterial source−sink phenomenon occurs with the volatile hydrocarbon, isoprene (2-methyl-1,3-butadiene). Isoprene emission has been detected from many different bacteria, especially bacilli (Kuzma *et al*., 1995; Wagner *et al*., 1999a), soils have been shown to contain significant levels of isoprene-consuming bacteria (Cleveland and Yavitt, 1997; 1998), and progress has been made in unravelling the pathway for isoprene degradation in *Rhodococcus* (van Hylckama Vlieg *et al*., 1998; 2000). This work may signal new interest in bacterial roles in biosphere−atmosphere exchange processes. Why is there

interest in biogenic isoprene? Isoprene is a reactive compound that is emitted to the atmosphere from terrestrial plants in large amounts, similar to global annual methane emissions (i.e. 500 million tons per year), and in smaller amounts from phytoplankton sources in the oceans (Guenther *et al*., 1995). Atmospheric chemists have been interested in the chemical reactions of isoprene for some time, and it is now known that, in the presence of sunlight and nitrogen oxides, isoprene reacts to form ozone, organic peroxides and carbon monoxide (Fehsenfeld *et al*., 1992). Indeed, in many ecosystems and even some urban areas, isoprene may dominate photochemical reactions in the atmospheric boundary layer (Chameides *et al*., 1988; Goldstein *et al*., 1998). Here, we review the emerging role of soil bacteria as sources or sinks of this important atmospheric hydrocarbon.

Bacterial sources of isoprene

The discovery of bacterial isoprene formation was an accidental finding that occurred during attempts to use expression cloning of a plant isoprene synthase gene (Kuzma, 1995). Control experiments showed that the *Escherichia coli* host used in these experiments formed a small amount of isoprene (in the head space of sealed Petri dishes), and then much larger amounts of isoprene were found in a contaminant that turned out to be a Gram-positive *Bacillus*. Screening of a variety of *Bacillus* species revealed that isoprene formation is very common in this genus; much smaller amounts of isoprene are released by several different Gram-negative bacteria (Kuzma *et al*., 1995). Isoprene formation by various actinomycetes, including *Pseudonocardia*, *Saccharomonospora*, *Streptomyces* and *Thermomonospora* isolates, has also been detected (Wilkins, 1996). Rates of isoprene formation in *Bacillus subtilis* are relatively high, similar to those seen in plants (on a cell weight basis), although when expressed as a fraction of glucose catabolism, isoprene is a minor cellular product (see below). On the other hand, rates of isoprene formation in *B. subtilis* are at least 100-fold higher than the rates of formation of the phytohormone, ethylene, produced by some bacteria, especially phytopathogens (Nagahama *et al*., 1992; Weingart and Völksch, 1997). Production of

isoprene in *B. subtilis* is highest in log-phase cells and occurs with a temperature optimum that indicates an enzymatic rather than a non-enzymatic source (Kuzma *et al.*, 1995); this is significant, as small amounts of isoprene are formed non-enzymatically in living cells from the breakdown of cellular dimethylallyl diphosphate (DMAPP) (Kuzma, 1995). Growth of *B. subtilis* under controlled conditions in a fermenter has revealed some surprising aspects of bacterial isoprene formation. First, using wild-type *B. subtilis* (Marburg strain), three distinct phases of isoprene release are seen in cells growing on a standard glucose–tryptone–salts medium, as shown in Fig. 1 (Wagner *et al.*, 1999). These three phases correspond to glucose catabolism, acetoin catabolism and the early stages of sporulation respectively. In phases 1 and 2, isoprene production is maximal during rapid assimilation of the available carbon source, and then drops rapidly to low levels between the phases. In phase 3, in which carbon sources are depleted and sporulation is initiated, isoprene release is maximal during prespore formation. In the experiment shown in Fig. 1, the total amount of isoprene formed (9 μmol) is only about 0.02% of the glucose consumed (50 mmol). It is not yet clear why this small, but significant, amount of volatile hydrocarbon is released under these growth conditions; possible reasons for bacterial isoprene production are discussed below. It should also be noted that more work will be needed to establish whether the patterns of isoprene formation seen in laboratory cultures (Fig. 1) have relevance to metabolic events occurring in soils. A second surprise was the finding that the pattern of isoprene formation in *B. subtilis* strain 168 is very different from that of its parent, the Marburg strain (Wagner, 1998). Strain 168, a tryptophan auxotroph with a stable *trpC2* mutation, was derived from the Marburg strain by X-ray mutagenesis (Burkholder and Giles, 1947; Dean

and Ziegler, 1989) and has been used extensively in the construction of genetic and physical maps of the *B. subtilis* chromosome (Anagnostopoulos *et al.*, 1993; Itaya, 1993) and for the *Bacillus* genome sequencing project (Kunst *et al.*, 1997). In experiments such as that shown in Fig. 1, strain 168 shows only the first phase of isoprene formation, even though it grows on acetoin and sporulates as well as the Marburg strain. This and other recent evidence (Wagner, 1998) suggest that strain 168 contains additional mutations that suppress the second and third phases of isoprene formation. Possibly, the chromosome of strain 168 underwent significant chromosomal rearrangements during its isolation from the Marburg strain (Itaya, 1997). Further work should reveal the nature of these other mutations in strain 168 and how they suppress phases 2 and 3 of isoprene formation.

Isoprene biosynthesis

The biosynthetic pathway for isoprene formation in *B. subtilis* has been studied, and it appears that isoprene is a product of the deoxyxylulose-5-phosphate (DXP) pathway (now also called the methylerythritol phosphate pathway), not the classical mevalonate pathway for isoprenoid biosynthesis (Wagner *et al.*, 2000). As reviewed by Eisenreich *et al.* (1998), in the past few years, it has become clear that many bacteria, both Gram-negative and Gram-positive, assemble the C-5 building blocks of isoprenoid biosynthesis, isopentenyl diphosphate (IPP) and DMAPP, from pyruvate and glyceraldehyde-3-phosphate, not from acetyl-CoA units. The condensation of pyruvate and glyceraldehyde-3-phosphate gives DXP, which can be reduced and rearranged via 2-*C*-methyl-ᴅ-erythritol-4-phosphate to produce IPP. In *B. subtilis*, ^{13}C-labelling experiments support the DXP pathway as the source of isoprene (Wagner *et al.*, 2000). The absence of the mevalonate pathway is also suggested by the findings that the *B. subtilis* genome lacks a gene for the key enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) (Bochar *et al.*, 1997), and the growth of *B. subtilis* is not blocked by simvastatin, a potent inhibitor of HMGR (Wagner *et al.*, 2000). The absence of the mevalonate pathway in *B. subtilis* is somewhat surprising, given that intermediates of this pathway have been detected in *Lactobacillus* and *Staphylococcus* (Horbach *et al.*, 1993); these bacteria are taxonomically similar to *Bacillus* in the low GC subdivision of Gram-positive bacteria (Woese, 1987). Although it seems likely that such closely related bacteria would use a common pathway for isoprenoid biosynthesis, perhaps some bacteria use more than one pathway, as suggested in the Gram-positive bacterium *Streptomyces aeriovifer* (Seto *et al.*, 1996).

   An enzyme that catalyzes DMAPP-dependent formation of isoprene has now been detected and partially purified
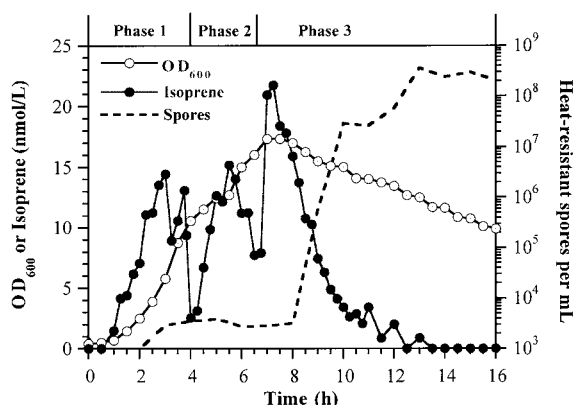

Fig. 1. Production of isoprene in wild-type *B. subtilis* 6051 occurs in three distinct phases. Phases 1 and 2 of isoprene formation occur during glucose and acetoin catabolism respectively; phase 3 occurs during the initial stages of sporulation. Data redrawn from Wagner *et al.* (1999a).

from *B. subtilis* (K. Choudhury and R. Fall, unpublished). Thus, is seems likely that isoprene is formed from DMAPP in *Bacillus*, as in the case of plant chloroplasts, which contain the enzyme isoprene synthase. Isoprene synthase catalyses the Mg^{2+}-dependent conversion of DMAPP to isoprene and PP_i (Silver and Fall, 1995; Wildermuth and Fall, 1996):
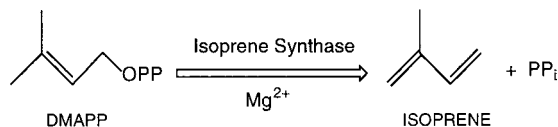
The isoprene synthase reaction involves simple elimination of pyrophosphate, probably through a transient carbocation that can rearrange to form isoprene. As in other isoprenoid synthases, the role of the divalent cation is to facilitate departure of the pyrophosphate-leaving group (McGarvey and Croteau, 1995). It remains to be seen whether there is a universal mechanism for isoprene formation in bacteria and plants, and also in humans, in whom isoprene is the major hydrocarbon released in expired breath (Stone *et al*., 1993).

Are there bacterial sinks for isoprene?

The question of bacterial sinks for isoprene has recently come into focus with the work of Cleveland and Yavitt (1997; 1998), who have shown that many soils contain isoprene-degrading microorganisms. Measuring the uptake of gas-phase isoprene (about 500 p.p.bv.), they determined that soils from many different ecosystems take up isoprene in a temperature-dependent manner, with little uptake in sterilized soil. The highest rates of isoprene uptake occurred in temperate forest soils and in soils pre-exposed to isoprene. For a temperate forest soil, levels of culturable isoprene degraders were in the range of 5.8×10^5 cfu g^{-1} dry weight, and enrichment cultures led to the identification of some of these microbes as *Arthrobacter* species. Previously, it has been shown that methanotrophs and a *Xanthobacter* isolate grown on propene were able to epoxidize isoprene and other alkenes, but not use isoprene as a carbon or energy source (Hou *et al*., 1981). Pure cultures of a *Nocardia* sp. were able to use isoprene or 1,3-butadiene as the sole source of carbon and energy (van Ginkel *et al*., 1987). Ewers *et al*. (1990) have also described isoprene-degrading bacteria that were used to co-metabolize trichloroethylene; they reported that isoprene-using bacteria are abundant in soil and water and characterized two of these isolates as *Alcaligenes denitrificans* ssp. *xylosoxidans* and *Rhodococcus erythropolis*. These reports and the work described below with another isoprene-degrading *Rhodococcus* suggest that the potential for metabolizing isoprene may be widespread in the Micrococcineae and Corynebacterineae suborders of the

actinomycetes (i.e. *Arthrobacter* and *Rhodococcus*). Interestingly, as noted above, some actinomycetes in other suborders produce isoprene, so the actinomycetes group of soil bacteria appears to contain both isoprene producers and degraders.

An estimate of a global annual soil sink for isoprene is 20.4 million tons year^{-1} (Cleveland and Yavitt, 1997), which is a small fraction (4%) of the estimated annual biogenic emission of isoprene (Guenther *et al*., 1995). However, it is not yet clear whether soil isoprene degraders can efficiently remove and consume isoprene from forest air. For example, levels of isoprene in canopy air at a high-isoprene, temperate oak forest site in the summer averaged 4.4 ppbv at midday, rarely exceeded 10 ppbv and dropped to 0.1–2 ppbv at night (Goldstein *et al*., 1998). Most of the decline in isoprene seen in the evening and night was attributed to chemical destruction of isoprene rather than deposition to the forest. Similar results have been seen at a mixed pine–oak–sweetgum forest site, although in this case, surface deposition of isoprene at night was proposed to be more significant (Goldan *et al*., 1995). Given these results, it is currently unclear whether gas-phase isoprene is a major source of carbon for soil bacteria. An alternative view is that aerobic isoprene degraders primarily consume isoprene generated in the soil by other bacteria. This source–sink relationship would be analogous to that seen with methanogens and methanotrophs. It is also possible that, under some circumstances, bacterial isoprene production might exceed degradation, and soils might then be a net source of isoprene. Few measurements of release of isoprene from soils (or sediments) have been reported (Guenther *et al*., 1995; Fall, 1999), so it is unclear whether soils contribute significantly to atmospheric isoprene.

The biochemical pathway for isoprene degradation

In recent work, the Janssen laboratory has unravelled many details of the biochemical pathway for isoprene degradation in *Rhodococcus* AD45, an isolate obtained from a freshwater sediment using isoprene as a sole carbon source (van Hylckama Vlieg *et al*., 1998; 1999; 2000; van Hylckama Vlieg, 1999). For this work, they used *Rhodococcus* AD45, an isolate obtained from a freshwater sediment enrichment with isoprene as sole carbon source. *Rhodococcus* species have the metabolic potential to degrade a variety of natural and man-made hydrocarbons (Larkin *et al*., 1998). *Rhodococcus* AD45 degrades 1,2-dichloroethylene, propylene, toluene and styrene, but with lower affinities and oxidation rates than those for isoprene (van Hylckama Vlieg, 1999). Figure 2 shows the pathway for isoprene degradation in *Rhodococcus* as far as it is known from biochemical and genetic experiments, as well as a proposal for further steps based upon the logical
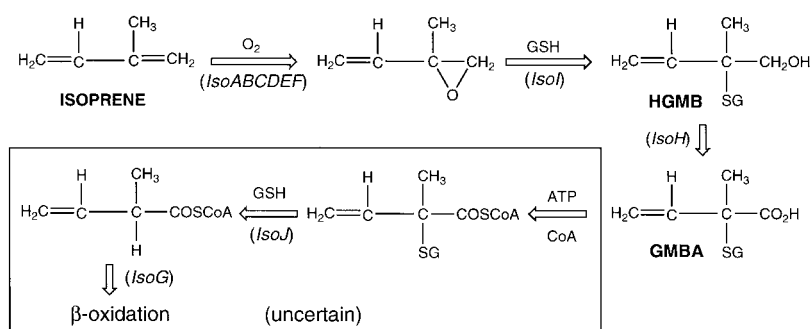
Fig. 2. Scheme for the degradation of isoprene in *Rhodococcus* AD45, based on information presented in van Hylckama Vlieg *et al.* (2000) and van Hylckama Vlieg (1999). The unknown portion of the pathway, enclosed in a box, is discussed in the text. The stereochemistry of the intermediates has not been established.

fate of the last characterized intermediate. The pathway begins with conversion of isoprene to 1,2-epoxy-2-methyl-3-butene, a reaction catalysed by a multicomponent monooxygenase encoded by genes *IsoABCDEF*. Although this enzyme system has not been purified, all six of the proteins encoded by this gene cluster are homologous to proteins in other characterized multicomponent monooxygenases (van Hylckama Vlieg, 1999). 1,2-Epoxy-2-methyl-3-butene is converted to a glutathione conjugate by a glutathione-*S*-transferase (GST) encoded by IsoI (van Hylckama Vlieg, 1999; van Hylckama Vlieg *et al.*, 1999). This reaction is a critical one, as it prevents the reactive epoxide from alkylating DNA and proteins. The enzyme has an unusually high K_M for glutathione ($> 5\,$mM), but is reasonably effective, nevertheless, with an estimated k_{cat}/K_M of $1.1 \times 10^4\,M^{-1}\,s^{-1}$ and a turnover number of $54\,s^{-1}$ at $5\,$mM glutathione (approximately twice the estimated intracellular concentration of glutathione in the bacterium). The glutathione conjugate, 1-hydroxy-2-glutathionyl-2-methyl-3-butene (HGMB), is oxidized in two steps to 2-glutathionyl-2-methyl-3-butenoic acid (GMBA) by IsoH, an NAD$^+$-requiring dehydrogenase. This enzyme appears to be unique and distinct from other known alcohol dehydrogenases, even the glutathione-dependent class III alcohol dehydrogenases (Barber *et al.*, 1996). It catalyses oxidation of the alcohol moiety in HGMB to the carboxylic acid, but is not active with a variety of other aliphatic primary or secondary alcohols (van Hylckama Vlieg *et al.*, 1999).

The sequence of steps after the formation of GMBA is not yet known. The reactions shown enclosed in a box in Fig. 2 are proposed based upon the following reasoning. The thioether bond of GMBA must ultimately be cleaved for catabolism to continue. There are two possible mechanisms for the cleavage reaction. The first, beta elimination of glutathione, is possible but unlikely, because it would be difficult to remove a proton from the inactivated methyl group and because thiolates are poor leaving groups. The second mechanism, reductive elimination of glutathione, would use a second glutathione molecule to reduce GMBA and form glutathione disulphide. This mechanism has a precedent in the reactions catalysed

by tetrachlorohydroquinone dehalogenase (another GST; McCarthy *et al.*, 1996; 1997), proline reductase (Arkowitz *et al.*, 1994) and glycine reductase (Arkowitz and Abeles, 1991). This reaction could be catalysed by IsoJ, a second GST encoded by the isoprene degradation gene cluster. Experiments from the Janssen laboratory have shown that IsoJ does not remove glutathione from GMBA (van Hylckama Vlieg, 1999), but it may certainly accomplish this reaction using a downstream metabolite.

A possible sequence for the remainder of the isoprene degradation pathway is that GMBA is converted to the corresponding CoA thioester by an unidentified ligase, before removal of glutathione by IsoJ (Fig. 2). This proposition is based upon the presence of an additional gene, *IsoG*, adjacent to *IsoH* in the isoprene degradation gene cluster (van Hylckama Vlieg, 1999), which is homologous to α-methylacyl-CoA racemases found in mammals (Schmitz *et al.*, 1997). This finding suggests that CoA thioester metabolites may be involved in the distal part of the pathway. Furthermore, the proposed reductive elimination of glutathione would be greatly facilitated by replacement of the carboxylate of HGMBA with a more electron-withdrawing thioester. Removal of glutathione from the CoA thioester of HGMBA would provide 2-methyl-3-butenyl CoA, which is an intermediate of the isoleucine degradation pathway (Massey *et al.*, 1976). Further metabolism of 2-methyl-3-butenyl CoA by a β-oxidation pathway would then yield acetyl-CoA and propionyl-CoA, which can be oxidized by central metabolic pathways. The role of the α-methylacyl-CoA racemase might be to facilitate complete degradation of methyl-substituted acyl-CoA intermediates. For example, in animals, the metabolism of branched-chain fatty acids requires racemization of the methyl group at the α-position for complete β-oxidation (Schmitz *et al.*, 1997). Similarly, it is known that oxidation of L-isoleucine occurs primarily through the (2*S*)-methylacyl-CoA intermediates (Sweetman and Williams, 1995) so, if isoprene catabolism in *Rhodococcus* ties into the isoleucine pathway, it may be necessary to convert a (2*R*)-methyl intermediate to its (2*S*)-methyl diastereomer.

Prospects

Many questions remain unanswered concerning bacterial isoprene synthesis and degradation. For example, why is isoprene formed, especially in Gram-positive bacteria? Is it a metabolic by-product or signalling molecule, or does it serve some other purpose? We have suggested that isoprene is an overflow metabolite (Wagner *et al*., 1999). It is well known that metabolic bottlenecks in bacteria lead to the excretion of a variety of extracellular metabolites (Neijssel and Tempest, 1979); for example, during aerobic culture of *B. subtilis* on glucose and amino acids, incomplete glucose oxidation results in the excretion into the medium of acetate, pyruvate, acetoin, butanediol and branched-chain organic acids (Speck and Freese, 1973). We envision that isoprene excretion could be a similar phenomenon that occurs under metabolic conditions in which carbon flow to higher isoprenoids is limiting and excess DMAPP is converted to isoprene. Quantitative assessment of (i) the rates of isoprenoid biosynthesis and (ii) isoprenoid pool sizes during different phases of *B. subtilis* growth will be needed to test this idea.

The possibility that bacterial isoprene formation serves some other more active role, such as in signalling, must also be considered. There are hints that isoprene could serve as a volatile repellent of soil predators or a growth inhibitor of competing microbes. For example, it has been reported that Collembola insects, which graze bacteria and fungi in soil, are repelled by isoprene (Michelozzi *et al*., 1997), and another C-5 volatile compound produced by some *Bacillus* spp., isoamyl alcohol, has growth-inhibiting effects on soil cyanobacteria (Wright *et al*., 1991). Perhaps isoprene release during rapid growth on available carbon sources is a way of inhibiting competitors when nutrients are abundant. In soil, it is known that *Bacillus* species are prevalent in the rhizosphere, where they can produce plant growth factors and synthesize antifungal agents (reviewed by Wipat and Harwood, 1999); it is possible that isoprene could play a role in these extracellular events. Finally, in terms of intercellular communication, it has been discovered that Gram-positive bacteria, including *B. subtilis*, use quorum sensing to control cell density-dependent signalling events (Kleerebezem *et al*., 1997), and it has been suggested that uncharacterized secondary metabolites may play a role in bacterial communication (Hastings and Greenberg, 1999). Isoprene might be such a secondary metabolite; its volatility would allow it to serve as a transient signalling molecule.

Studies of the role of isoprene formation in *Bacillus* could shed light on the mystery of isoprene production in higher organisms (Fall, 1999; Logan and Monson, 1999). Currently, there is no clear role for isoprene formation in plants or humans and, as mentioned in the *Introduction*, the global release of isoprene by plants represents a very large amount of reduced carbon. Any model for leaf isoprene emission needs to explain developmental control of isoprene formation during leaf expansion, chloroplast localization of isoprene synthase isozymes, regulation of DMAPP levels in chloroplasts and the role of light activation of isoprene formation (Fall and Wildermuth, 1998). Similarly, the physiological rationale for release of isoprene in human breath is unknown, although a link to cholesterol biosynthesis has experimental support (Stone *et al*., 1993; Taucher *et al*., 1997). Possibly, studies with the *B. subtilis* system, with its rich background of genetic and genomic information (Anagnostopoulos *et al*., 1993; Wipat and Harwood, 1999), will reveal a rationale for isoprene formation in living systems.

The detection of soil bacteria that can use isoprene as sole carbon source suggests that soil may be a significant sink for this gas. There is current interest in accounting for isoprene sinks in forest canopies, so additional characterization of isoprene degraders is important. Do these bacteria consume isoprene generated in soil, or do they take up atmospheric isoprene? In this regard, it will be interesting to determine the affinity of the isoprene monooxygenase for isoprene; the K_m for oxidation of isoprene by intact *Rhodococcus* cells is 0.8 μM (van Hylckama Vlieg *et al*., 1998). Levels of the hydrocarbon in forest canopies, even those dominated by isoprene-emitting species, rarely exceed 10 p.p.bv. (Goldstein *et al*., 1998). As the Henry's law constant for isoprene is about 0.028 M atm^{-1} (Lindinger *et al*., 1998), concentrations of isoprene in soil surface water in equilibrium with forest air would be less than 1 nM, probably too low to support growth of bacteria unless they are remarkably efficient in scavenging and concentrating isoprene. It seems more likely that isoprene degraders use isoprene formed *in situ*.

A particularly interesting aspect of the isoprene degradation pathway in *Rhodococcus* is the involvement of two members of the GST superfamily. The identification of GSTs involved in isoprene degradation adds to the growing body of information suggesting that the major role of GSTs in bacteria is metabolism rather than detoxification, the primary role of GSTs in mammals. Other bacterial GSTs involved in metabolic pathways include maleylacetoacetate isomerase, tetrachlorohydroquinone dehalogenase, β-etherase and dichloromethane dehalogenase (Seltzer, 1973; LaRoche and Leisinger, 1990; Masai *et al*., 1993; McCarthy *et al*., 1996). IsoI, the first GST in the isoprene degradation pathway, catalyses the simple nucleophilic attack of glutathione upon its substrate to form a glutathione conjugate, a typical GST reaction. The role of the second GST, IsoJ, may be analogous to those of two other members of the superfamily, tetrachlorohydroquinone dehalogenase and β-etherase, which catalyse reductive reactions, resulting in the formation of glutathione disulphide. However, a notable difference is

that glutathione conjugates are both formed and reduced at the active site of tetrachlorohydroquinone dehalogenase (McCarthy *et al*., 1997), and presumably of β-etherase (Masai *et al*., 1993), but the glutathione conjugate in the isoprene pathway is formed several steps before its anticipated reduction. The reasons for this difference are easily understood, as reductive elimination of glutathione from the initially formed conjugate is energetically unfeasible because of the lack of an electron-withdrawing group at the alpha position to stabilize the negatively charged intermediate. Thus, the isoprene catabolic pathway provides an interesting variation upon the usual themes of detoxification and substrate reduction by members of the GST superfamily.

It will be of interest to see the completed enzymatic details of the catabolic pathway for isoprene in *Rhodococcus* and the relationship of this pathway to those for degradation of other natural and anthropogenic hydrodrocarbons. With work on recruiting catabolic genes for use in pollutant degradation by engineered microorganisms under way, it is likely that analysis of the gene products of the *Rhodococcus* isoprene pathway will be revealing; for example, several of the enzymes of the pathway have the potential to use chlorinated substrate analogues (van Hylckama Vlieg, 1999; van Hylckama Vlieg *et al*., 1998, 1999).

With the study of methanogens and methanotrophs, a wealth of basic biochemistry was revealed (e.g. Ferry, 1997). Time will tell if biochemical analysis of isoprene biosynthesis and degradation in bacteria will yield such a rich harvest. Whatever the outcome, the information is sure to be of interest to microbiologists and atmospheric scientists alike.

Acknowledgements

References

Anagnostopoulos, C., Piggot, P.J., and Hoch, J.A. (1993) The genetic map of *Bacillus subtilis*. In Bacillus subtilis *and Other Gram-Positive Bacteria. Biochemistry, Physiology, and Molecular Biology*. Sonenshein, A.L., Hoch, J.A., and Losick, R. (eds). Washington, DC: American Society of Microbiology Press, pp. 425–461.

Arkowitz, R.A., and Abeles, R.H. (1991) Mechanism of action of clostridial glycine reductase: isolation and characterization of a covalent acetyl-enzyme intermediate. *Biochemistry* 30: 4090–4097.

Arkowitz, R.A., Dhe-Paganon, S., and Abeles, R.H. (1994) The fate of the carboxyl oxygens during D-proline reduction by clostridial proline reductase. *Arch Biochem Biophys* 311: 457–459.

Barber, R.D., Rott, M.A., and Donohue, T.J. (1996) Characterization of a glutathione-dependent formaldehyde dehydrogenase from *Rhodobacter sphaeroides*. *J Bacteriol* 178: 1386–1393.

Bochar, D.A., Brown, J.R., Doolittle, W.F., Klenk, H.P., Lam, W., Schenk, M.E., *et al*. (1997) 3-Hydroxy-3-methylglutaryl coenzyme A reductase of *Sulfolobus solfataricus*: DNA sequence, phylogeny, expression in *Escherichia coli* of the *hmg*A gene, and purification and kinetic characterization of the gene product. *J Bacteriol* 179: 3632–3638.

Burkholder, P.R., and Giles, N.H. (1947) Induced biochemical mutations in *Bacillus subtilis*. *Am J Bot* 33: 345–348.

Chameides, W.L., Lindsay, R.W., Richardson, J., and Kiang, C.S. (1988) The role of biogenic hydrocarbons in urban photochemical smog: Atlanta as a case study. *Science* 241: 1473–1475.

Cleveland, C.C., and Yavitt, J.B. (1997) Consumption of atmospheric isoprene in soil. *Geophys Res Lett* 24: 2379–2382.

Cleveland, C.C., and Yavitt, J.B. (1998) Microbial consumption of atmospheric isoprene in a temperate forest soil. *Appl Environ Microbiol* 64: 172–177.

Conrad, R. (1996) Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). *Microbiol Rev* 60: 609–640.

Crutzen, P.J. (1991) Methane's sinks and sources. *Nature* 350: 380–381.

Dean, D.H., and Ziegler, D.R. (1989) *Bacillus Genetic Stock Center. Strains and Data*, 4th edn. Columbus, OH: The Ohio State University.

Eisenreich, W., Schwarz, M., Cartayrade, A., Arigoni, D., Zenk, M.H., and Bacher, A. (1998) The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chem Biol* 5: R221–R233.

Ewers, J., Clemens, W., and Knackmuss, H.-J. (1990) Selection of trichloroethylene (TCE) degrading bacteria that resist inactivation by TCE. *Arch Microbiol* 154: 410–413.

Fall, R. (1999) Biogenic emissions of volatile organic compounds from higher plants. In *Reactive Hydrocarbons in the Atmosphere*. Hewitt, C.N. (ed.). San Diego, CA: Academic Press, pp. 41–96.

Fall, R., and Wildermuth, M.C. (1998) Isoprene synthase: from biochemical mechanism to emission algorithm. *J Geophys Res* 103: 25599–25609.

Fehsenfeld, F., Calvert, J., Fall, R., Goldan, P., Guenther, A.B., Hewitt, C.N., *et al*. (1992) Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Global Biogeochem Cycles* 6: 389–430.

Ferry, J.B. (1997) Methane: small molecule, big impact. *Science* 278: 1413–1414.

Goldan, P.D., Kuster, W.C., Fehsenfeld, F.C., and Montzka, S.A. (1995) Hydrocarbon measurements in the southeastern United States: the Rural Oxidants in the Southern Environment (ROSE) program 1990. *J Geophys Res* 100: 25945–25963.

Goldstein, A.H., Goulden, M.T., Munger, J.W., Wofsy, S.C., and Geron, C.D. (1998) Seasonal course of isoprene emissions from a midlatitude deciduous forest. *J Geophys Res* 103: 31045–31056.

Guenther, A., Hewitt, C.N., Erickson, D., Fall, R., Geron, C., Graedel, T., *et al*. (1995) A global model of natural volatile organic compound emissions. *J Geophys Res* 100: 8873–8892.

Hanson, R.S., and Hanson, T.E. (1996) Methanotrophic bacteria. *Microbiol Rev* 60: 439–471.

Hastings, J.W., and Greenberg, E.P. (1999) Quorum sensing: the explanation of a curious phenomenon reveals a common characteristic of bacteria. *J Bacteriol* 181: 2667–2668.

Horbach, S., Salm, H., and Welle, R. (1993) Isoprenoid biosynthesis in bacteria: two different pathways. *FEMS Microbiol Lett* **111:** 135–140.

Hou, C.T., Patel, R.N., Laskin, N., Barnabe, N., and Barist, I. (1981) Epoxidation and hydroxylation of C4- and C5-branched-chain alkenes and alkanes by methanotrophs. *Ind Microbiol* **23:** 477–482.

Itaya, M. (1993) Physical map of the *Bacillus subtilis* 168 chromosome. In Bacillus subtilis *and Other Gram-Positive Bacteria. Biochemistry, Physiology, and Molecular Biology*. Sonenshein, A.L., Hoch, J.A., & Losick, R. (eds). Washington, DC: American Society for Microbiology PRESS, pp. 463–471.

Itaya, M. (1997) Physical map of the *Bacillus subtilis* 166 genome: evidence for the inversion of an approximately 1900 kb continuous DNA segment, the translocation of an approximately 100 kb segment and the duplication of a 5 kb segment. *Microbiology* **143:** 3723–3732.

King, G.M. (1992) Ecological aspects of methane oxidation, a key determinant of global methane dynamics. *Adv Microb Ecol* **12:** 431–468.

Kleerebezem, M., Quadri, L., Kuipers, O., and Vos, W.d. (1997) Quorum sensing by peptide hormones and two-component signal transduction systems in Gram-positive bacteria. *Mol Microbiol* **24:** 895–904.

Kunst, K., Ogasawara, N., Mosze, I., Albertini, A.M., Alloni, G., Azevedo, V., *et al.* (1997) The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* **390:** 249–256.

Kuzma, J. (1995) *Regulation of Isoprene Production from Plants and Microorganisms*. PhD Thesis, University of Colorado.

Kuzma, J., Nemecek-Marshall, M., Pollock, W.H., and Fall, R. (1995) Bacteria produce the volatile hydrocarbon isoprene. *Curr Microbiol* **30:** 97–103.

Larkin, M.J., Mot, R.D., Kulakov, L.A., and Nagy, I. (1998) Applied aspects of *Rhodococcus* genetics. *Antonie van Leeuwenhoek Int J Gen Mol Microbiol* **74:** 133–153.

LaRoche, S.D., and Leisinger, T. (1990) Sequence analysis and expression of the bacterial dichloromethane dehalogenase structural gene, a member of the glutathione-*S*-transferase supergene family. *J Bacteriol* **172:** 164–171.

Lindinger, W., Hansel, A., and Jordan, A. (1998) On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS). Medical applications, food control and environmental research. *Int J Mass Spectr Ion Proc* **173:** 191–241.

Logan, B.A., and Monson, R.K. (1999) Thermotolerance of leaf discs from four isoprene-emitting species is not enhanced by exposure to exogenous isoprene. *Plant Physiol* **120:** 821–825.

McCarthy, D.L., Navarrete, S., Willett, W.S., Babbitt, P.C., and Copley, S.D. (1996) Exploration of the relationship between tetrachlorohydroquinone dehalogenase and the glutathione *S*-transferase superfamily. *Biochemistry* **35:** 14634–14642.

McCarthy, D.L., Louie, D.F., and Copley, S.D. (1997) Identification of a covalent intermediate between glutathione and cysteine 13 during catalysis by tetrachlorohydroquinone dehalogenase. *J Am Chem Soc* **119:** 11337–11338.

McGarvey, D.J., and Croteau, R. (1995) Terpenoid metabolism. *Plant Cell* **7:** 1015–1026.

Masai, E., Katayama, Y., Kubota, S., Kawai, S., Yamasaki, M., and Morohoshi, N. (1993) A bacterial enzyme degrading the model lignin compound, β-etherase, is a member of the glutathione-*S*-transferase superfamily. *FEBS Lett* **323:** 135–140.

Massey, L.K., Sokatch, J.R., and Conrad, R.S. (1976) Branched-chain amino acid catabolism in bacteria. *Bacteriol Rev* **40:** 42–54.

Michelozzi, M., Raschi, A., Tognetti, R., and Tosi, L. (1997) Eco-ethological analysis of the interaction between isoprene and the behavior of Collembola. *Pedobiologia* **41:** 210–214.

Nagahama, K., Ogawa, T., Fujii, T., and Fukuda, H. (1992) Classification of ethylene-producing bacteria in terms of biosynthetic pathways to ethylene. *J Ferment Bioeng* **73:** 1–5.

Neijssel, O.M., and Tempest, D.W. (1979) The physiology of metabolite over-production. In *Microbial Technology: Current State, Future Prospects*. Bull, A.T., Ellwood, D.C., and Ratledge, C. (eds). Cambridge: Cambridge University Press, pp. 53–82.

Potter, C.S., Davidson, E.A., and Verchot, L.V. (1996) Estimation of global biogeochemical controls and seasonality in soil methane consumption. *Chemosphere* **32:** 2219–2246.

Schmitz, W., Helander, H.M., Hiltunen, J.K., and Conzelmann, E. (1997) Molecular cloning of cDNA species for rat and mouse liver methylacyl-CoA racemases. *Biochem J* **326:** 883–889.

Seltzer, S. (1973) Purification and properties of maleylacetone *cis-trans* isomerase from *Vibrio* 01. *J Biol Chem* **248:** 215–222.

Seto, H., Watanabe, H., and Furihata, K. (1996) Simultaneous operation of the mevalonate and non-mevalonate pathways in the biosynthesis of isopentenyl diphosphate in *Streptomyces aeriouvifer*. *Tetrahedron Lett* **37:** 7979–7982.

Silver, G.M., and Fall, R. (1995) Characterization of aspen isoprene synthase, an enzyme responsible for leaf isoprene emission to the atmosphere. *J Biol Chem* **270:** 13010–13016.

Speck, E.L., and Freese, E. (1973) Control of metabolite secretion in *Bacillus subtilis*. *J Gen Microbiol* **78:** 261–275.

Stone, B.G., Besse, T.J., Duane, W.C., Evans, C.D., and DeMaster, E.G. (1993) Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men. *Lipids* **28:** 705–708.

Sweetman, L., and Williams, J.C. (1995) Branched chain organic acidurias. In *The Metabolic Basis of Inherited Disease*. Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D. (eds). New York: McGraw-Hill, pp. 1387–1422.

Taucher, J., Hansel, A., Jordan, A., Fall, R., Futrell, J.H., and Lindinger, W. (1997) Detection of isoprene in expired air from human subjects using proton-transfer-reaction mass spectrometry. *Rapid Commun Mass Spectr* **11:** 1230–1234.

van Ginkel, C.G., Jong, E.D., Tilanus, J.W.R., and Bont, J.A.M. (1987) Microbial oxidation of isoprene, a biogenic foliage volatile and of 1,3-butadiene, an anthropogenic gas. *FEMS Microbiol Ecol* **45:** 275–279.

van Hylckama Vlieg, J.E.T. (1999) *Reactive Epoxides as Intermediates in the Bacterial Metabolism of Isoprene and Chlorinated Ethenes*. PhD Thesis, Groningen University.

van Hylckama Vlieg, J.E.T., Kingma, J., van den Wijngaard, A.J., and Janssen, D.B. (1998) A glutathione *S*-transferase with activity towards *cis*-1,2-dichloroepoxyethane is involved in isoprene utilization by *Rhodococcus* sp. strain AD45. *Appl Environ Microbiol* **64:** 2800–2805.

van Hylckama Vlieg, J.E.T., Kingma, J., Kruizinga, W., and Janssen, D.B. (1999) Purification of a glutathione S-transferase and a glutathione conjugate-specific dehydrogenase involved in isoprene metabolism in *Rhodococcus* sp. strain AD45. *J Bacteriol* **181:** 2094–2101.

van Hylckama Vlieg, J.E.T., Leemhuis, H., Lutje Spelberg, J.H., and Janssen, D.B. (2000) Characterization of the gene cluster involved in isoprene metabolism in *Rhodococcus* sp. strain AD45. (in press).

Wagner, W.P. (1998) *Isoprene Production in* Bacillus. MS Thesis, University of Colorado.

Wagner, W.P., Nemecek-Marshall, M., and Fall, R. (1999) Three distinct phases of isoprene formation during the growth and sporulation of *Bacillus subtilis*. *J Bacteriol* **181:** 4700–4703.

Wagner, W.P., Helmig, D., and Fall, R. (2000) Isoprene biosynthesis in *Bacillus subtilis* via the methylerythritol phosphate pathway. *J Nat Prod* **63:** 37–40.

Weingart, H., and Völksch, B. (1997) Ethylene production by *Pseudomonas syringae* pathovars *in vitro* and *in planta*. *Appl Environ Microbiol* **63:** 156–161.

Wildermuth, M.C., and Fall, R. (1996) Light-dependent isoprene emission. Characterization of a thylakoid-bound isoprene synthase in *Salix discolor*. *Plant Physiol* **112:** 171–182.

Wilkins, K. (1996) Volatile metabolites from actinomycetes. *Chemosphere* **32:** 1427–1434.

Wipat, A., and Harwood, C.R. (1999) The Bacillus subtilis genome sequence: the molecular blueprint of a soil bacterium. *FEMS Microbiol Ecol* **28:** 1–9.

Woese, C.R. (1987) Bacterial evolution. *Microbiol Rev* **51:** 221–271.

Wright, S.J.L., Linton, C.J., Edwards, R.A., and Drury, E. (1991) Isoamyl alcohol (3-methyl-1-butanol), a volatile anti-cyanobacterial and phytotoxic product of some *Bacillus* spp. *Lett Appl Microbiol* **13:** 130–132.